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# Are there pathways for protein folding?

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**Abstract**

Denatured proteins, which have had essentially all of their native three-dimensional structure disrupted, can refold from their random disorderd state into a well-defined unique structure, in which the biological activity is virtually completely restored. This experimental result has lead to the suggestion that a native protein exists in some kind of thermodynamic configurational equilibrium, with the biologically active state being the one of lowest configurational energy. An alternative view is that the native protein is in a uniquely selected metastable state, in which the configurational energy is at a local minimum but not necessarily at an absolute minimum. In this latter model, the protein is not assumed to be in an equilibrium state, and one must postulate some sequence of events which takes place for each molecule so that the protein reaches the correct metastable state.

One possible sequential process which might lead a protein to land in a particular state, is the growth of the peptide chain on the ribosome, starting with the amino terminal end and preceding to the carboxyl terminus. Although one could imagine a protein folding as it grows, and thus attaining a particular metastable state, this is clearly not a necessary condition for correct folding, at least for those proteins which have been shown to be reversibly denaturable. However, the fact that folding on the ribosomes is not necessary for the establishment of structure, does not imply that any theory invoking a pathway of folding can be eliminated. Such a pathway only requires some local initiations or condensations of segments of the polypeptide chain whenever the denatured protein is put into the appropriate renaturing medium. These segments would form unique three-dimensional structures which make further condensation more likely. Thus, a pathway of folding means that there exist a well-defined sequence of events which follow one another so as to carry the protein from the unfolded random coil to a uniquely folded metastable state. If the final folded state turned out to be the one of lowest configurational energy, it would be a consequence of biological evolution not of physical chemistry.

Three approaches have been used in investigating this problem. First, the refolding and dimerzation of the enzyme alkaline phosphatase obtained from the bacterium E. Coli has been studied under varying conditions and from a variety of mutant strains. Mutants have been selected which fail to make active enzyme at 44 °C. About half of these mutants have activity when the cells are grown at 25 °C, and the enzyme produced at the low temperature has been found to be stable even at temperatures much higher than that used in the selection. Thus, these mutants have a temperature-sensitive step in one of the events which normally leads to the formation of active enzyme, but the enzyme produced is not temperature sensitive.

A second approach involved the use of computer- aided molecular model building in attempts to deduce plausible pathways which proteins can follow as they are folding. Starting with an amino acid sequence we can describe the configuration of the protein i.e., the position of each of its atoms in space if we know the dihedral angles for the backbone and, in addition, the rotation angles about the appropriate bonds of the amino acid residues. Using a computer controlled display system, the molecule thus generated can be displayed in such a way that the observer can see the three-dimensional relationships in the structure. Computer programs have been written in such a way that any configuration can be altered to minimize the Van der Vaals energy and to insure close packing of the structure. However, this energy minimization can only be expected to alter the structure to the bottom of the local minimum; it is not intended to search through all possible configurations for a true minimum energy. In addition, the investigator can alter the computer generated structure as if he were dealing with physical models in which one part could be pushed or pulled relative to another. Thus, the computer- aided model building is not designed to find the configuration of minimum energy rather, it is designed as an aid to the investigator as various sequentially folding steps are tried.

This system has been used in an attempt to obtain such a pathway of folding for the protein cytochrome C. A plausible structure has been obtained in this way which satisfies all of the known chemical interactions of the molecule. However, the uniqueness of the proposed folding process has not been determined.

Finally, the computer system has been used in attempts to deduce plausible folding pathways for myoglobin and lysozyme. Three-dimensional pictures of the structures and some of the folding sequences will be shown.